

AMENDED CLAIMS

AP3 Rec'd PCT/PTO 15 JUN 2005

[Received by the International Bureau on 08 July 2005 (08.07.2005):
original pages 50, 51, 52, 54, 57, 58 and 59 replaced by amended pages 50, 51, 52, 54, 57,
58 and 59]

WHAT IS CLAIMED IS:

1. A method of cell culture comprising:
confining a cell between first and second barriers, said barriers spaced at a distance comparable to the size of said cell to contact said cell and prevent said cell from traveling toward or away from each of said first and second barriers;
placing one or more spacers between said first and second barriers to prevent said first and second barriers over-compressing said cell; and
providing to said cell a culture substance.
2. The method of claim 1 wherein said first barrier is urged toward abutment with said second barrier and wherein said confining and said placing comprise at least partially separating said first and second barriers and introducing said cell and said one or more spacers between said first and second barriers.
3. The method of claim 1 or claim 2 wherein said one or more spacers are rigid spheres, each having a diameter comparable to the size of said cell.
4. The method of any one of claims 1 to 3 wherein each of said barriers has one or more characteristics selected to mimic the characteristics of the biological niche of said cell.
5. The method of any one of claims 1 to 4, wherein said providing comprises establishing a pre-selected concentration gradient of said culture substance within said space.
6. The method of any one of claims 1 to 5, further comprising manually positioning a specific cell type adjacent said cell.
7. The method of any one of claims 1 to 6, wherein said first and second barriers comprise first and second plates.
8. The method of any one of claims 1 to 7, wherein a monolayer of cells are cultured between said barriers.
9. The method of claim 8, further comprising removing a cell from said monolayer of cells when said cell to be removed is observed to meet one or more pre-determined criteria.

10. The method of claim 9, wherein said one or more criteria are related to one or more of karyotype, morphology, and size.
11. The method of any one of claims 1 to 10 further comprising:
limiting the number of cells surrounding said cell to permit control of properties of said cell.
12. The method of claim 11 wherein said cells surrounding said cell are selected to provide selected surfaces adjacent said cell.
13. The method of claim 2 wherein said introducing comprises introducing a suspension of said cell and said one or more spacers between said first and second barriers.
14. The method of claim 2 or claim 13 further comprising fixing ends of said second barrier to said first barrier with an elastic fixative, said elastic fixative urging said first barrier toward abutment with said second barrier.
15. The method of any one of claims 1 to 14 wherein said cell is confined between a surface of said first barrier and a surface of said second barrier, each surface selected to limit adhesion of said cell to said each surface.
16. The method of any one of claims 1 to 15 wherein said one or more spacers are configured to, with said first and second barriers, define a space having a shape mimicking the shape of the biological niche of said cell.
17. The method of any one of claims 1 to 16, wherein said providing comprises flowing said culture substance to said cell at a predetermined rate.
18. The method of claim 7, wherein said plates are optically transparent.
19. The method of any one of claims 1 to 18, wherein said providing comprises allowing said culture substance to permeate through one or both of said first and second barriers to between said first and second barriers.

20. The method of any one of claims 1 to 19 further comprising sensing, with a sensor disposed adjacent a space between said first and second barriers, one or more of molecular concentration, temperature, osmolarity, pH, and shear force.

21. The method of claim 15 wherein said surface of said first barrier comprises a first type of molecules and said surface of said second barrier comprises a second type of molecules.

22. A method of forming an artificial tissue, comprising laying a first monolayer of cells on a second monolayer of cells and permitting cells of said first monolayer to interact with cells of said second monolayer wherein each one of said first and second monolayers is cultured according to the method of any one of claims 1 to 21.

23. A combination of cell culture device and cell culture comprising:

first and second barriers and one or more spacers there-between defining a desired cell culture space said barriers contacting cells or bodies of cells constrained in said culture space wherein the spacers are sufficiently rigid to resist movement of said first and second barriers and approximate the size of said cells or bodies of cells such that the distance between said first and second barriers is comparable to the size of the cells or bodies of cells to be cultured therein and the spacers prevent said first and second barriers from overly compressing said cells or bodies of cells;

and means for providing to said culture space a culture substance at a predetermined rate.

24. The combination of claim 23 wherein said means for providing to said space a culture substance comprises one or more fluid passageways allowing fluid communication to and from said space.

25. The combination of claim 24, wherein said one or more fluid passageways comprise one or more microfluidic channels each terminating adjacent said space.

26. The combination of any one of claims 23 to 25 further comprising means for regulating fluid flow to or from said space.

38. The combination of any one of claims 23 to 37 wherein said one or more spacers are removable from said first or second barrier.
39. The combination of any of claims 23 to 37 wherein said one or more spacers are molded on said first or second barrier.
40. The combination of claims 23 to 37, further comprising a divider for dividing said chamber into a plurality of regions and for preventing said cell from moving between said regions.
41. The combination of claim 40, wherein said divider is removable from said container.
42. The combination of any one of claims 23 to 41, wherein the surfaces of said barriers comprise different types of molecules.
43. The combination of any one of claims 23 to 42, comprising a permeable membrane positioned to cover an opening adjacent said space for preventing said cell from leaving said space through said opening.
44. The combination of any one of claims 23 to 43, wherein said barriers defining a plurality of spaces for confining a plurality of cells therebetween.
45. The combination of any one of claims 23 to 44, which is included in a cartridge.
46. The combination of any one of claims 23 to 45, further comprising said fluid culture medium which is received in said chamber and immerses said cell.
47. The combination of claim 46, wherein at least one wall of said container has a septum allowing access to said space with a syringe or a pipette.
48. The combination of any one of claims 23 to 47, further comprising a capillary conduit for transporting a cell-containing fluid to or from said space.

69. The combination of claim 68, wherein said divider is removable from said container.
70. The combination of any one of claims 49 to 69, wherein the surfaces of said barriers comprise different types of molecules.
71. The combination of any one of claims 49 to 70, comprising a permeable membrane positioned to cover an opening adjacent said space for preventing a cell from leaving said space through said opening.
72. The combination of any one of claims 49 to 71, wherein said barriers defining a plurality of spaces for confining a plurality of assembly of cells therebetween.
73. The combination of any one of claims 49 to 72, which is included in a cartridge.
74. The combination of any one of claims 49 to 73, further comprising said fluid culture medium which is contained in said chamber and immerses said assembly of cells.
75. The combination of claim 74, wherein at least one wall of said container has a septum allowing access to said space with a syringe or a pipette.
76. The combination of any one of claims 49 to 75, further comprising a capillary conduit for transporting a fluid to or from said space.
77. An assembly of cells comprising a cell cultured in accordance with the method of any one of claims 1 to 21.
78. A cell cultured in accordance with the method of any one of claims 1 to 21.
79. The assembly of cells of claim 77 or the cell of claim 78 for use as an artificial tissue, organ, cell transplant, or *in vitro* fertilization.
80. A apparatus for culturing cells in a controlled environment comprising:
- i) first and second barriers and one or more spacers there-between defining a desired confined space wherein the distance between the barriers is comparable to the size of cells or bodies of cells to be cultured therein, the barriers contacting said cells or bodies of cells impeding their movement wherein said spacers are sufficiently rigid to resist movement of said first and second barriers;

and prevent first and second barriers from overly compressing said cells or bodies of cells.

ii) the inner surface of one or both of said first and second barriers having one or more characteristics or properties selected to mimic the characteristics of the biological environment of said cells;

iii) means for providing a culture substance to said space.

81. The apparatus of claim 80 wherein said barriers comprise two opposing glass plates.

82. The apparatus of claim 80 or claim 81, wherein said space has a size comparable to the size of a single cell.

83. The apparatus of any one of claims 80 to 82, wherein said space limits cells cultured therein to a monolayer.

84. The apparatus of any one of claims 80 to 83 wherein said means for providing comprises one or more fluid passageways allowing fluid communication to and from said space.

85. The apparatus of claim 84 wherein said one or more fluid passageways comprise one or more microfluidic channels terminating adjacent said space.

86. The apparatus of any one of claims 80 to 85 further comprising means for regulating fluid flow to or from said space.

87. The apparatus of any one of claims 80 to 86 wherein at least one of said barriers is permeable to nutrients and gases.

88. The apparatus of any one of claims 80 to 87 further comprising means for monitoring a cell constrained in said space.

89. The apparatus of claim 88 wherein said means for monitoring comprises a sensor disposed in said chamber.

90. The apparatus of claim 89 wherein said sensor is a sensor for sensing one or more of molecular concentration, temperature, osmolarity, pH, and shear force.
91. The apparatus of claim 89 or claim 90, further comprising one or more transparent electrodes for connecting said sensor to a control system.
92. The apparatus of any one of claims 80 to 91, wherein at least a portion of one of said barriers is transparent.
93. The apparatus of claim 92, wherein one of said barriers is a microscope cover slip.
94. The apparatus of claim 93, wherein said portion of said barrier is made of one of polystyrene, porous glass, or other contact lens materials.
95. The apparatus of any one of claims 80 to 94, wherein at least one of said barriers is moveable for adjusting the size of said space.
96. The apparatus of claim 95, further comprising an actuator for moving said at least one of said barriers.
97. The apparatus of claim 96, wherein said actuator comprises one or more of an inflatable bladder, a screw, a lever, a clamp, a micrometer, and a piezoelectric crystal.
98. The apparatus of any one of claims 80 to 97 wherein said one or more spacers are removable from said first or second barrier.
99. The apparatus of any one of claims 80 to 97 wherein said one or more spacers are molded on said first or second barrier.
100. The apparatus of claims 80 to 98, further comprising a divider for dividing said chamber into a plurality of regions and for preventing said cell from moving between said regions.
101. The apparatus of claim 99, wherein said divider is removable from said container.